Free radical formation during the oxidation of 2'-7'-Dichlorofluorescin. Possible implications for measurement of reactive oxygen and nitrogen species

Christina Rota[1], Aldo Tomasi[1] and Ronald P. Mason[2]

[1]Department of Biomedical Science, University of Modena and Reggio Emilia, Via Campi 287 41100 Modena, Italy
[2]Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences, National Institutes of Health, RTP, North Carolina, 27709, USA

The fluorogenic probe 2'-7'-dichlorofluorescin (DCFH) is widely used for assaying the formation of reactive oxygen and nitrogen species in cells and tissues, via its oxidation to the fluorescent dye 2'-7'-dichlorofluorescein (DCF). In previous studies we demonstrated free radical formation during the photoreduction of DCF and peroxidase catalyzed oxidation of DCFH or DCF [1].

In the present study we analyzed the oxidation of DCFH by horseradish peroxidase (HRP) in the presence of peroxynitrite ($ONOO^-$). Visible spectroscopy showed the reduction of HRP-compound I to compound II upon addition of 1 μM DCFH to a mixture containing 1 μM $ONOO^-$ and 1 μM HRP, demonstrating the occurrence of an one-electron oxidation, with the obligate formation of the $DCF^{\bullet}$ semiquinone free radical. Fluorescence development at 522 nrn was observed in a system containing 5 μIM DCFH, 150 μM GSH and 1 μM $ONOO^-$, fluorescence level was still very intense in the absence of GSH, it was HRP dependent and strongly catalase inhibitable. A very intense fluorescence level was also found after preincubation of peroxynitrite in buffer for as long as 30 minutes, in this case fluorescence was completely inhibited by catalase, implying the formation of a peroxide-like compound. When Electron Spin Resonance (ESR) spectroscopy in conjunction with the spin trapping technique was employed, $^{\bullet}OH$, $GS^{\bullet}$ and $^{\bullet}OOH$ free radical adducts were detected in the complete system, $^{\bullet}OH$ resulted to be the major species. In the absence of DCFH, $GS^{\bullet}$ was the most abundant species detected. Superoxide dismutase (SOD) and catalase showed inhibitory effect. The same radical species (with lower intensity) were detected when peroxynitrite was preincubated for 30 minutes: also in this case, SOD and catalase had inhibitory effect.

Our results strongly demonstrate that a large amount of artifacts can arise from the usage of this probe and other structurally similar fluorogenic probes.

[1]. Rota, C., Fann, Y. C., and Mason, R. P. *J. Biol. Chem.* **274**,28161-68,1999